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Comparison of AlloDerm and AlloMax Tissue Incorporation in Rats

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Background: Human acellular dermal matrices (HADMs) are used in a variety of settings. AlloMax is a new HADM currently being used for breast reconstruction and hernia repair. We compared the in vivo tissue integration of AlloMax to AlloDerm, a well-studied HADM, in rats.

Methods: We implanted AlloDerm and AlloMax patches into subcutaneous pockets on the backs of 32 male Sprague-Dawley rats. The animals were killed after either 4 or 8 weeks, and the patches were recovered and stained for histopathologic analyses. Microscopic end points included patch thickness, vascularization, tissue in-growth, fibroblast proliferation, and inflammation.

Results: All animals completed the study without complications or infection. There were no significant differences in graft thicknesses at 4 and 8 weeks. Microscopically, at 4 weeks, AlloDerm sections had significantly more microvessels than AlloMax ($P = 0.02$). This disparity increased by 8 weeks ($P < 0.01$). Similarly, we found greater tissue in-growth and fibroblast proliferation in AlloDerm than AlloMax sections at 4 ($P < 0.01$) and at 8 ($P < 0.01$) weeks. Inflammatory infiltrates consisted of lymphocytes, histiocytes, eosinophils, and plasma cells. Deep graft infiltration by predominately lymphocytic inflammatory cells was significantly higher in AlloDerm than AlloMax grafts at 4 ($P = 0.01$) and 8 ($P = 0.02$) weeks. Graft necrosis was uncommon, but marginal fibrosis was similar in both.

Conclusions: AlloDerm grafts had greater neovascularization, tissue infiltration, fibroblast proliferation, and inflammatory reaction than AlloMax grafts when placed subcutaneously in rats. AlloDerm may be better incorporated than AlloMax when placed in vivo.

Key Words: AlloDerm, AlloMax, human acellular dermis, biocompatible materials, collagen, tissue scaffolds, materials testing, regenerative medicine

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Human acellular dermal matrices (HADMs) have been in clinical use for more than 15 years and are used in a variety of reconstructive procedures. Surgeons may now choose from several HADMs that, at first glance, appear remarkably similar. All HADMs are derived from cadaveric tissue, must undergo decellularization to prevent immunogenic rejection, and appear to promote tissue ingrowth with neovascularization after implantation. Despite these similarities, each HADM has distinguishing properties stemming from the various processing methods for the cadaveric tissue. These proprietary decellularization and sterilization processes lead to different storage requirements, shelf lives, intraoperative preparation, and cost. What matters most though is actual product performance, and most HADMs lack comparative clinical and experimental evidence of their capabilities.

The purpose of our study was to compare the tissue incorporation of a new HADM, AlloMax (CR Bard/Davol Inc, Cranston, RI), to the widely used AlloDerm (LifeCell Corp, Branchburg, NJ). AlloDerm has been well studied in both animal experiments and human clinical research. Previous animal studies have shown that AlloDerm promotes tissue integration and neovascular-ization.[1–6] Clinical reports have shown that AlloDerm can provide satisfactory cosmetic outcomes in a variety of reconstructive procedures.[7–10] Other studies, though, have raised concerns over the performance of AlloDerm. Because AlloDerm is not terminally sterilized during processing, controversy has arisen regarding increased infection rates in sterile procedures such as breast reconstruction.[11] AlloDerm has also developed a reputation for excessive laxity resulting in recurrent hernias when used for abdominal wall reconstruction.[8,12] Other HADM products such as AlloMax have been developed to address these issues. To counter concerns of contamination, AlloMax is one of the 2 currently marketed HADMs to undergo terminal sterilization. AlloMax also claims to cause less stretch. Do the unique AlloMax preparation processes, however, inhibit in vivo tissue incorporation? Our study is the first to compare the in vivo performance of AlloMax with AlloDerm.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee at David Grant USAF Medical Center. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 86–23, revised 1996). All animal handling and research were conducted in our facility, which is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. No author was affiliated with Lifecell or Bard.

Animal Handling and Preparation

We used 32 adult male Sprague-Dawley rats, weighing [mean (SEM)] 431 (24) g. The animals were fed a commercial diet and observed for 10 days before initiating the study. Food was withheld for 1 hour before surgery to ensure that food material was not present in the mouth. The rats were initially anesthetized with 5% isoflurane in oxygen at 1 L/min in an induction chamber. Anesthesia was maintained with 2% isoflurane in 1 L/min oxygen delivered by face mask to effect. After clipping the hair over the rat's dorsum, the skin was prepared by alternating 3 chlorhexidine scrub and sterile saline rinses.

Surgical Procedure

AlloMax and AlloDerm grafts of comparable thickness (both listed as 0.8–1.8 mm) were used. Despite the similar thickness listed on the packaging, the AlloMax we used grossly appeared thicker than AlloDerm. The grafts were prepared according to the manufacturer's instructions during surgery. AlloDerm was rehydrated in a 2-step process. The AlloDerm was first placed in a warm saline bath until the paper backing separated from the AlloDerm. The material was then submerged for at least 15 minutes in another saline bath at which point the graft was soft and pliable. AlloMax was rehydrated by

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Subject category: Wound healing/plastic surgery.

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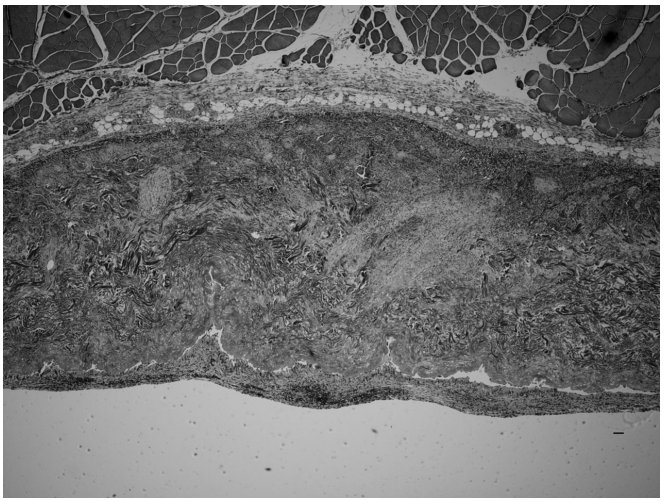

FIGURE 1. AlloDerm cross-section 4 weeks after implantation. Note the extensive tissue infiltration and inflammatory response. Masson trichrome stain, original magnification ×4. Bar = 50 μ.

submerging the implant in a saline solution for 5 minutes, at which point the material was supple and pliable. The surgery was performed in a manner similar to the previous study of Richter et al[5] evaluating dermal matrices in rats. A 4-cm midline dorsal skin incision was made. Flaps were raised between the panniculus carnosus and the deep muscular fascia. A piece of AlloDerm was fashioned into a 1 × 1-cm patch and placed on the right side of the pocket. Care was taken to ensure that the dermal side faced the underlying muscular fascia. A piece of AlloMax was identically fashioned and placed on the left side of the pocket. The implants were not sutured in place to prevent any inflammatory reaction from the suture material interfering with the results. The fascia was closed with 4-0 polyglactin 910 suture (Vicryl; Ethicon, Inc, Somerville, NJ), and the skin was closed with 5-0 poliglecaprone 25 (Monocryl; Ethicon, Inc) in a running subcuticular pattern. Cyanoacrylate adhesive (Vetbond; 3M Inc, Minneapolis, MN) was used to reinforce the skin closure. After surgery, the rats were maintained in a warm environment until completely recovered from the procedure, at which time they were returned to their normal housing. The rats received buprenorphine at 0.01 to 0.05 mg/kg subcutaneously every 12 hours for 48 hours and then ad libitum.

Data Collection

Half of the rats were killed at 4 weeks, whereas the remaining rats were killed at 8 weeks. Rats were euthanized by carbon dioxide inhalation in accordance with the 2007 AVMA Guidelines on Euthanasia. The implanted materials were removed en bloc and preserved in 10% formalin before histologic processing. Sections were cut from the center of each graft, sectioned at 4 μm, and routinely processed with hematoxylin and eosin and Masson trichrome stains. Measurements of graft thickness were carried out with image analysis software (Image Pro Express v 6.0; Media Cybernetics, Bethesda, MD). Histologic analyses were adapted from previously reported methods,[4,5] including the mean number of microvessels in 3 high-power fields from the most vascular areas on each slide, the estimated area of tissue in-growth on each slide, and the estimated area occupied by fibroblasts on each slide. Each slide was also evaluated by a pathologist (B.C.) blinded to time point and material, who reviewed sections for the presence of necrosis, fibrosis, surrounding granulation tissue, foreign body giant cell reaction, and type and location of inflammatory cells. Fibrosis was defined as dense collagen and fibroblastic reaction not associated with suture material. The presence of granulation tissue was assessed in a semiquantitative fashion as none, one focus, or multiple foci, to include presence of circumferential vascular proliferation and inflammation surrounding the graft. The location of leukocytes was evaluated based on presence of leukocytes at the periphery, superficial infiltration, or deep infiltration of the graft. Because peripheral inflammation and superficial infiltration were difficult to separate in practice, these 2 categories were combined. The graft was only considered to have deep infiltration by leukocytes if the cells were seen in the center of the graft, not associated with probable fibrosis and reaction secondary to suture material.

Statistical Analysis

The primary outcomes were neovascularization, tissue infiltration, and degree of inflammation. All data are presented as mean (SEM). Analysis of variance was used for numerical data analysis, and count data were analyzed with χ^2 statistics, both conducted with commercial statistical software (STATA v10; StataCorp, College Station, Tx), with $P < 0.05$ being considered statistically significant.

RESULTS

All rats survived until the time of planned kill. No gross signs of infection or implant rejection occurred in any rat; all surgical wounds appeared well healed. During the implant harvest, the AlloDerm and AlloMax implants were readily identifiable and incorporated with the surrounding tissue at both 4 and 8 weeks. In situ, blood vessels could be seen crossing both graft materials by 4 weeks. Mean (SEM) graft thicknesses for AlloDerm were 1.33 (0.08) mm at 4 weeks and 1.7 (0.06) mm at 8 weeks ($P = 0.56$). Comparable values for AlloMax were 1.5 (0.06) and 1.6 (0.05), respectively ($P = 0.48$; see Figure 1 and Figure 2 for representative sections). The overall graft appearance was unaltered from the time of implantation; histologic evaluation, however, showed significant differences between the 2 materials.

Figure 3 indicates that neovascularization was evident in both AlloDerm and AlloMax patches at 4 weeks, although AlloDerm had significantly greater neovascularization than AlloMax at 4 and 8 weeks ($P = 0.02$ and $P < 0.01$, respectively). By 8 weeks, AlloDerm

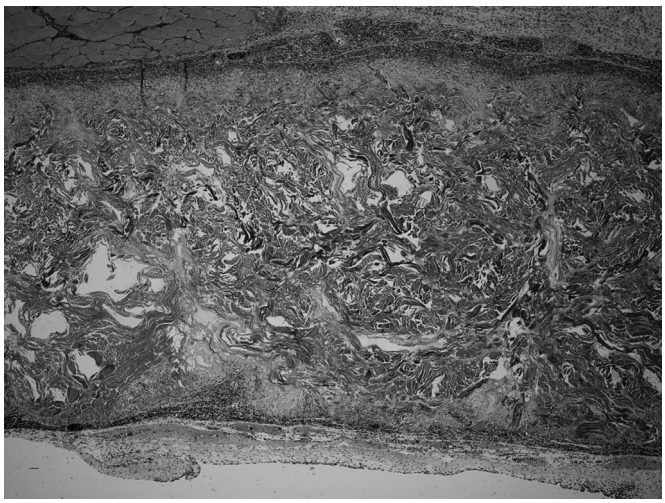

FIGURE 2. AlloMax cross-section 4 weeks after implantation. Note the minimal tissue infiltration and marginal inflammatory response. Masson trichrome stain, original magnification ×4. Bar = 50 μ.

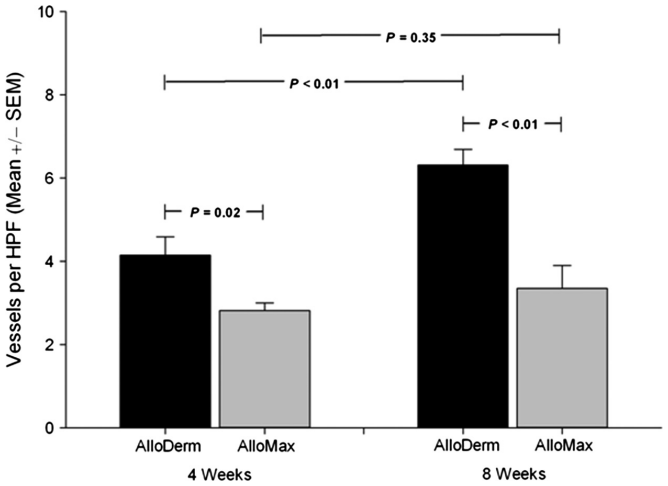

FIGURE 3. Mean number of vessels per high-power field.

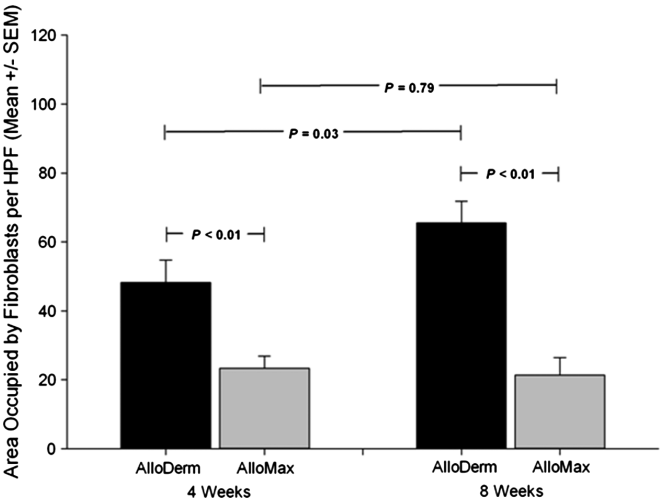

FIGURE 5. Mean area occupied by fibroblasts per high-power field.

patches had significantly more microvessels than what were present at 4 weeks ($P < 0.01$). However, this same trend was not observed in the AlloMax patches, and there were no significant differences in neovascularization of 4- and 8-week AlloMax patches ($P = 0.35$).

AlloDerm patches also had significantly more cellular infiltration (see Fig. 4) compared with AlloMax patches at 4 and 8 weeks ($P < 0.01$). AlloDerm patches also showed a significant increase in cellular infiltration between 4 and 8 weeks ($P < 0.01$), whereas the cellularity of AlloMax patches remained essentially the same between the 2 time points ($P = 0.53$). In addition, the estimated area occupied by fibroblasts in the AlloDerm patches (see Fig. 5) was significantly greater at 4 and 8 weeks than in the AlloMax patches ($P < 0.01$). The area occupied by fibroblasts increased significantly between 4 and 8 weeks in the AlloDerm patches ($P = 0.03$), whereas the area occupied by fibroblasts declined in the AlloMax patches, although not significantly ($P = 0.79$).

Necrosis was only observed in 1 AlloDerm graft, at 8 weeks. In the 4-week grafts, fibrosis was observed in 0 (0%) of 16 AlloDerm grafts and 3 (18.8%) of 16 AlloMax grafts ($P = 0.82$). In the 8-week grafts, fibrosis was observed in 11 (68.8%) of 16 AlloDerm grafts and 8 (50%) of 16 AlloMax grafts ($P = 0.28$). Foreign body giant cell reaction was identified in all AlloDerm and nearly all AlloMax grafts at both 4 and 8 weeks. All AlloDerm and AlloMax grafts at both 4 and 8 weeks had superficial inflammatory infiltrates consisting of lymphocytes, histiocytes, eosinophils, and plasma cells. Deep infiltration of the grafts by lymphocyte-predominant mixed inflammatory cells was observed in 10 (62.5%) of 16 AlloDerm and 3 (18.8%) of 16 AlloMax grafts at 4 weeks ($P = 0.01$). Deep infiltration of the zgrafts by lymphocyte-predominant mixed inflammatory cells was observed in 5 (31.2%) of 16 AlloDerm grafts and 0 (0%) of 16 AlloMax grafts at 8 weeks ($P = 0.02$). Although the presence of inflammatory cells was similar in both groups at 4 weeks, by 8 weeks, AlloDerm exhibited significantly more inflammatory cells than AlloMax, mainly because of the decrease in cells in the AlloMax group. The presence of eosinophils was similar for both groups at 4 and 8 weeks.

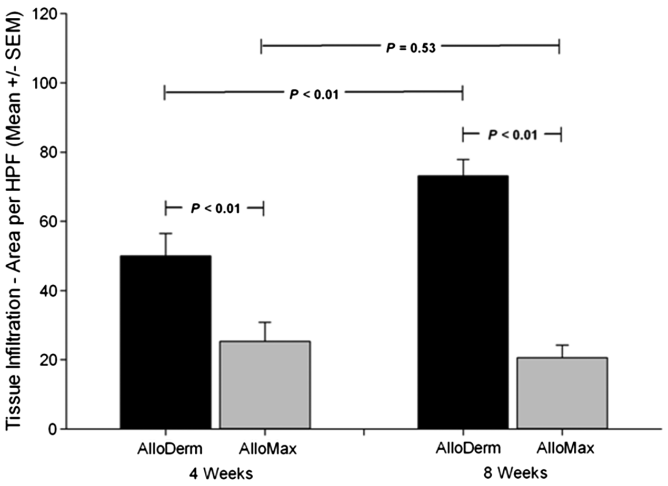

FIGURE 4. Mean percent area with tissue infiltration per high-power field.

DISCUSSION

Our study showed that AlloMax stimulated tissue in-growth and neovascularization. This tissue incorporation, however, was limited and did not appear to increase after 4 weeks. By contrast, AlloDerm tissue integration appeared more pronounced than AlloMax at 4 weeks and continued to progress at 8 weeks. Neither material caused any complications.

Since AlloDerm first appeared on the market in 1995, several other HADM have been developed including FlexHD (Ethicon Inc, Somerville, NJ), DermaMatrix (Synthes Inc, West Chester, PA), and AlloMax. These competitors claim a few advantages over AlloDerm, including less intraoperative preparation time and the truly sterile nature of their product. AlloDerm itself has evolved over the years. Per current handling instructions, AlloDerm no longer needs to be refrigerated during storage, and its applications have broadened from skin grafting to other procedures such as breast and abdominal wall reconstruction. Despite these improvements, some questions remain over the clinical performance of AlloDerm implants. A recent study cited increased wound infections when using AlloDerm compared with other breast reconstruction techniques, and several reports cite the propensity for hernia recurrence and abdominal wall laxity with AlloDerm.[8,11,12] AlloDerm use is also associated with "red breast syndrome," a sterile, chronic cellulitis that resolves without treatment but results in clinical confusion and patient discomfort. This may be

due to the AlloDerm preparation process, inflammatory mediators from neovascularization, or simply reversing the proper orientation of the product at implantation (ie, placing the dermal side on the least vascularized tissue).[13–15] Although other HADMs have also been associated with red breast syndrome, its occurrence has not been documented in NeoForm or AlloMax.

AlloMax addresses these concerns by undergoing a terminal sterilization process, claiming less ex vivo elasticity than AlloDerm, and not mandating a specific polarity (and thus preventing a possible cause of red breast syndrome). It is unknown, though, if the AlloMax preparation processes inhibit native tissue integration. Even when dermal matrices share a common source, the proprietary preparation processes may greatly affect in vivo tissue integration and performance.[16] Different graft preparation methods (such as collagen cross-linking) can trigger different host immune responses, resulting in either reconstructive remodeling or chronic inflammation.[16] Nonetheless, other studies show little difference in tissue incorporation despite using dermal matrices from different species and undergoing different preparation processes.[1,3,4,7] Nonetheless, prominent clinical issues such as AlloDerm in vivo laxity after remodeling may be anticipated by animal studies such as ours.[4,17]

NeoForm, the only other terminally sterilized HADM available, has shown promising initial results when used in breast reconstruction, but no studies until now have provided evidence of in vivo tissue integration of AlloMax.[18] The only other studies discussing AlloMax showed a marked discrepancy in human macrophage response and cytokine production in comparison with AlloDerm in vitro. AlloMax generated a significantly greater amount of interleukin (IL) 1β, IL-8, IL-6, and VEGF.[19] These in vitro results would suggest a greater in vivo inflammatory response to AlloMax. This increased cytokine production could either lead to a chronic inflammatory response with encapsulation or more robust remodeling. Our study suggests that AlloMax processing may retard tissue remodeling when compared with AlloDerm. The agents used to decellularize AlloMax may account for these differences.[20] A review article by Crapo et al[21] provides an overview of how these different agents can alter biologic materials and possibly inhibit in vivo remodeling. Sodium hydroxide can damage collagen and remove growth factors. Acetone, which also is used for delipidation, may cross-link collagen. Finally, the gamma radiation used to sterilize the product may damage the extracellular matrix as well. The preparation methods for AlloDerm, however, may also affect its performance.[20,21] Sodium deoxycholate can remove glycosaminoglycans, and freeze drying may damage the extracellular matrix. Whatever the key discrepancy is, our study shows that AlloMax promotes less tissue remodeling than AlloDerm. Although the AlloMax grafts we used also grossly appeared thicker than AlloDerm, the lack of tissue remodeling in the AlloMax grafts suggests that the thickness was not the reason for these differences.

There are several possible clinical implications with these findings. The lack of ongoing remodeling suggests that AlloMax may eventually become encapsulated with collagen like other foreign bodies and remain unabsorbed. The recent emphasis on using HADM as biologic scaffolds for regenerative medicine would make AlloMax a less suitable material than AlloDerm. The comparative lack of neovascularization would also render AlloMax less infection resistant compared with other biologic implants such as AlloDerm. The relative paucity of cellular infiltration, however, may have its benefits. The relatively rapid reabsorption of AlloDerm may make it less suitable for reconstructive procedures where durability is essential, such as abdominal wall reconstruction. Facial reconstructive procedures where a preserved contour is desired may also warrant a more durable material than AlloDerm.[17] AlloMax may thus provide greater tensile strength and be prone to less stretching than AlloDerm, although we did not test these properties in our study. With the increased presence of inflammatory cells in AlloDerm, studies taken beyond an 8-week end point could show AlloMax to be a more durable material. Regardless, AlloMax and AlloDerm performance in humans may be closer than what our animal study suggests. Despite the rigorous decellularization and sterilization processes used, some HADMs still retain some donor DNA and antigens.[22] This may retard tissue integration in other species but provide less impedance in humans. Implantation longer than 8 weeks in humans may show further cellular infiltration of AlloMax, although our results with rats showed a lack of tissue remodeling after 4 weeks of implantation. Further studies must be done examining the tissue integration, infection resistance, and clinical application of AlloMax.

In conclusion, AlloMax does promote tissue integration of native tissue with minimal to no recipient site morbidity. The in vivo tissue integration of AlloDerm, however, is significantly greater than AlloMax in rats, and AlloMax's tissue integration appears static 4 weeks after implantation.

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